



Supplemental Figure 6

**Supplemental Figure 6. CD4<sup>+</sup> T cells promote carcinogenesis upon Treg depletion.** (A) Pancreas to body weight ratio and spleen to body weight ratio of KC, KC;Foxp3<sup>DTR</sup> vehicle control and KC;Foxp3<sup>DTR</sup> CCR1 inhibitor treated. Data represent mean  $\pm$  SEM, n=5-6. The statistical difference was determined by two-tailed t-tests. (B) Quantification of CK19 or Amylase positive area (%) in co-immunofluorescent staining for KC;Foxp3<sup>DTR</sup> vehicle control and CCR1 inhibitor treated pancreata. Data represent mean  $\pm$  SEM, n=3-4. (C) Quantification of CD45 positive area (%) and F4/80 positive area (%) in co-immunofluorescent staining for control and CCR1 inhibitor treated KC;Foxp3<sup>DTR</sup> pancreata. Data represent mean  $\pm$  SEM, n=3. (D) Flow cytometry analysis for CD3<sup>+</sup>CD4<sup>+</sup>IFN $\gamma$ <sup>+</sup> T cells, CD3<sup>+</sup>CD4<sup>+</sup>IL4<sup>+</sup> T cells from WT, Foxp3<sup>DTR</sup>, KC, KC;Foxp3<sup>DTR</sup> and KC;CD4<sup>-/-</sup> mice that received DT for 1 week starting 8 weeks post pancreatitis induction. Data is shown as percentage of total leukocytes or percentage of total cells. Data represent mean  $\pm$  SE; the statistical difference between experimental groups was determined by two-tailed t-tests. (E) qRT-PCR for *Il2*, *Ifn $\gamma$* , *Tnf $\alpha$* , *Il17 $\alpha$* , *Il22*, *Il4*, *Il5*, *Il10*, *Il13* and *Tgf $\beta$ 1* expression in T cells sorted from the pancreas of KC and KC;Foxp3<sup>DTR</sup> mice (n=5-6). Data represent mean  $\pm$  SEM; the statistical difference between experimental groups was determined by two-tailed t-tests. (F) Quantification of PanIN formation in KC and KC;Foxp3<sup>DTR</sup> mice that received DT treatment and/or anti-CD4 antibody is shown as lesion number per 10X field, n=3. (G) CD45<sup>+</sup> leukocytes, CD3<sup>+</sup> T cells, CD3<sup>+</sup>CD4<sup>+</sup> T cells, CD3<sup>+</sup>CD4<sup>+</sup>FoxP3<sup>+</sup> Tregs, CD3<sup>+</sup>CD8<sup>+</sup> T cells, CD11b<sup>+</sup> myeloid cells, CD11b<sup>+</sup>F4/80<sup>+</sup> macrophages and CD11b<sup>+</sup>F4/80<sup>+</sup>Ly6C<sup>+</sup>/Ly6G<sup>+</sup> MDSCs from pancreata were measured by flow cytometry as percentage of total cells. Data represent mean  $\pm$  SEM, n=2-3; the statistical difference between experimental groups was determined by two-tailed t-tests.